*Risk Analysis, Vol. 37, No. 2, 2017* DOI: 10.1111/risa.12603

# Modeling U-Shaped Exposure-Response Relationships for Agents that Demonstrate Toxicity Due to Both Excess and Deficiency

**Brittany Milton,[1,*] Patrick J. Farrell,[1,3] Nicholas Birkett,[2,3] and Daniel Krewski[1,2,3]**

Essential elements such as copper and manganese may demonstrate U-shaped exposure-response relationships due to toxic responses occurring as a result of both excess and deficiency. Previous work on a copper toxicity database employed CatReg, a software program for categorical regression developed by the U.S. Environmental Protection Agency, to model copper excess and deficiency exposure-response relationships separately. This analysis involved the use of a severity scoring system to place diverse toxic responses on a common severity scale, thereby allowing their inclusion in the same CatReg model. In this article, we present methods for simultaneously fitting excess and deficiency data in the form of a single U-shaped exposure-response curve, the minimum of which occurs at the exposure level that minimizes the probability of an adverse outcome due to either excess or deficiency (or both). We also present a closed-form expression for the point at which the exposure-response curves for excess and deficiency cross, corresponding to the exposure level at which the risk of an adverse outcome due to excess is equal to that for deficiency. The application of these methods is illustrated using the same copper toxicity database noted above. The use of these methods permits the analysis of all available exposure-response data from multiple studies expressing multiple endpoints due to both excess and deficiency. The exposure level corresponding to the minimum of this U-shaped curve, and the confidence limits around this exposure level, may be useful in establishing an acceptable range of exposures that minimize the overall risk associated with the agent of interest.

**KEY WORDS:** Copper toxicity; deficiency; excess; severity scoring; U-shaped exposure-response curve

## 1. INTRODUCTION

The establishment of a daily recommended intake of an essential nutrient requires determination both of the minimum intake that will satisfy nutritional requirements and the maximum tolerable intake that will not result in toxicity.[1] Because too much or too little of any such nutrient can be harmful to human health, the challenge is to define an allowable range of oral intakes that will not result in adverse health outcomes due to either excess or deficiency. The modeling of exposure-response relationships for both excess and deficiency provides the foundation for meeting this challenge.

Historically, the U.S. Environmental Protection Agency (U.S. EPA) developed the concept of a reference dose (RfD) for toxic substances, which has been widely accepted and used in practice. The RfD is defined as the level of oral intake of

[1]Carleton University, School of Mathematics and Statistics, Ottawa, Ontario, Canada.
[2]School of Epidemiology, Public Health and Population Medicine, Ottawa, Ontario, Canada.
[3]RS McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, Ontario, Canada.
*Address correspondence to Brittany Milton, Carleton University, School of Mathematics, and Statistics, Ottawa, Ontario, Canada; brittany_milton@carleton.ca.


0272-4332/17/0100-0265$22.00/1 © 2016 Society for Risk Analysis

ED_002191_00008780-00001

a substance that can be ingested over an extended period of time without appreciable risk of an adverse health outcome. It is usually derived from a single key study that considers one critical health effect. The RfD is obtained through the application of adjustment factors to the no-observed-adverse-effects level (NOAEL), which corresponds to the level of exposure that does not result in a significant increase in the risk of adverse effects in the exposed group when compared with controls.[2] The benchmark dose (BMD), which is the dose corresponding to a specified increase in risk[3] (usually in the range of 1–10%), has also been used as a point of departure on the dose-response curve for establishing human exposure guidelines.[4] More recently, the signal-to-noise crossover dose (SNCD), defined as the dose at which the uncertainty in the biological signal is indistinguishable from the background noise has been introduced as an alternative to the BMD.[5] Categorical regression has found application in dose-response modeling for risk assessment purposes.[6–8] By assigning severity scores to different health endpoints, categorical regression facilitates their inclusion in a single exposure-response model.

Chambers *et al.*[8] used U.S. EPA CatReg[9] software to conduct a categorical regression analysis using the copper toxicity database developed by Krewski *et al.*[10] Using this tool, Chambers *et al.*[8] were able to fit exposure-response curves for Cu excess and deficiency separately, and identified an optimal copper intake level of 2.6 mg/day. The current U.S. recommended dietary intake (RDI) is 0.9 mg/day, while the tolerable upper intake level is 10 mg/day.[1] Of the procedures noted above for establishing human exposure guidelines, the RfD relies to a considerable extent on expert opinion. This includes specification of the most critical effect, which may be subject to toxicological interpretation. While similar issues arise with the BMD, the main concern is that several exposure-response models could appropriately characterize the observed data, but could lead to different risk predictions outside the range of the available data. Perhaps the most limiting feature of the RfD, BMD, and SNCD approaches is that the safe exposure level ultimately relies on one critical health effect from a single study. Categorical regression addresses this issue by using all of the available data, including data from different studies with different endpoints, in a single analysis. Chambers *et al.*[8] applied categorical regression to the copper toxicity database noted above, but considered excess and deficiency separately, and used a splicing procedure to combine the excess and deficiency exposure-response curves.

In this article, a joint model for excess and deficiency—the JMED—is proposed. The JMED is defined so that the dose and origin of toxicity (excess or deficiency) predict the probability of an adverse health effect. We present a closed-form solution for the point at which the excess and deficiency curves in this joint model cross. We present a second model based on the assumption that the toxicological processes leading to excess and deficient responses are independent, with the component excess and deficiency curves based on the JMED. Identifying the optimal intake level from this curve, defined as the dose that minimizes the probability of an adverse response due to either excess or deficiency (or both), requires the use of numerical optimization. The remainder of this article focuses on the theoretical development of these two models, and their practical application in describing U-shaped dose-response curves for copper excess and deficiency.

## 2. METHODS

### 2.1. Categorical Regression Applied to Toxicological Data

Categorical regression is a statistical tool that can be used to estimate the probability of an adverse health outcome associated with health risks from exposure to toxic substances.[10] These health effects are assigned to ordinal severity categories: a simple three-category severity scoring system, for example, might represent no effect (severity 0), a mild effect (severity 1), or a more severe effect (severity 2). This tool is appealing to risk assessors because it facilitates the use of multiple independent variables, such as concentration and duration of exposure, to describe multiple adverse outcomes through the use of a common categorical severity metric.

Although categorical regression is a powerful tool for toxicological risk analysis,[11] it too is subject to limitations. Toxicological judgment is needed to rate the severity of the observed effects and to categorize the effects across multiple studies in a consistent manner.[12] Although a model may appear to fit the data well, there is no way to evaluate the accuracy achieved in extrapolation beyond the range of the available data.[12] Finally, categorical regression is statistically driven: no information on

ED_002191_00008780-00002

the biological processes underlying the induction of adverse health outcomes is used in developing the model.

Categorical regression involves an ordinal response variable. In the general case, the response is composed of $S$ ordered categories. In developing the concept of categorical regression, it is helpful to begin with the special case in which the response falls into one of two categories ($S = 2$), with or without order being assumed.

## 2.2. Binary Logistic Regression

Consider a vector of random variables, $\mathbf{Y}$, which can each take on one of two possible values, coded 0 or 1. Given a data set of $n$ independent observations, $\mathbf{Y}$ can be considered a column vector of $n$ Bernoulli random variables that can be described using a binary logistic regression model.[14] For the $i^{\text{th}}$ observation ($i = 1,.., n$), $Y_i = 1$ represents a "success" in the Bernoulli sense (the occurrence of an adverse outcome in the present application), with $Y_i = 0$ representing a "failure" (here, the absence of an adverse outcome). As in any regression model, there is an underlying assumption that the response variable is related to one or more of $K$ explanatory variables, $x_{1i}, \ldots, x_{Ki}$, according to a specified functional relationship. For the case of the Bernoulli random variable $Y_i$, its associated success parameter $\pi_i$, is modeled as $P(Y_i = 1|x_{1i}, \ldots, x_{Ki}) = \pi_i$, with $P(Y_i = 0|x_{1i}, \ldots, x_{Ki}) = 1 - \pi_i$, and is referred to as the link function. The link function is often taken to be the logistic function since it is easily converted to a linear (or nonlinear) relationship by the *logit* transformation.[14] For illustrative purposes, we will assume a linear relationship. The logistic function defines $P(Y_i = 1|x_{1i}, \ldots, x_{Ki})$ as:

$$\pi_i = \frac{\exp(\beta_0 + \beta_1 x_{1i} + \cdots + \beta_K x_{Ki})}{1 + \exp(\beta_0 + \beta_1 x_{1i} + \cdots + \beta_K x_{Ki})}, \quad (1)$$

where $\pi_i \in (0, 1)$ and $\beta^T = [\beta_0, \beta_1, \ldots, \beta_K]$ is a vector of regression parameters consisting of an intercept term, $\beta_0$, and slope parameters $\beta_1, \ldots, \beta_K$. The logistic regression model equates the logit transform to the linear component:

$$\text{logit}(\pi_i) = \ln\left(\frac{\pi_i}{1 - \pi_i}\right) = \sum_{k=0}^{K} \beta_k x_{ki} = \beta^T \mathbf{X}, \quad (2)$$

where $\mathbf{X}$ is an $n \times (K + 1)$ matrix containing a column vector of 1s followed by the vectors $\mathbf{x}_i^T = [x_{1i}, x_{2i}, \ldots, x_{Ki}]$.

### 2.2.1. Parameter Estimation

The goal of binary logistic regression is to estimate the $K+1$ unknown parameters in Equation (2), which can be done using the method of maximum likelihood.[13,14] The maximum likelihood estimator is based on the probability distribution of the dependent variable, $\mathbf{Y}$. Since each $Y_i$ represents a Bernoulli random variable for the $i^{\text{th}}$ observation and these $Y_i$ are independent, the likelihood of the data is:

$$\mathcal{L}(\beta|\mathbf{y}) = \prod_{i=1}^{n} \pi_i^{y_i} (1 - \pi_i)^{1-y_i}, \quad (3)$$

where $\pi_i$ is defined in Equation (1) and $y_i$ is the observed value of $Y_i$, taking on a value of either 0 or 1. The maximum likelihood estimates (MLEs) are the values of $\beta$ that maximize the likelihood function in Equation (3). The critical points of a function (maxima and minima) occur when the first derivative equals zero. If the second derivative evaluated at that point is less than zero, then the critical point is a maximum. Finding the MLEs requires computation of the first and second derivatives of the likelihood function. This procedure is simplified by using the log-likelihood function, which is simply attained by applying the natural log transform to Equation (3), giving:

$$l = \log \mathcal{L} = \sum_{i=1}^{n} y_i \log(\pi_i) + \sum_{i=1}^{n} (1 - y_i) \log(1 - \pi_i). \quad (4)$$

Maximality will be preserved since the logarithm is a monotonic function. To obtain the MLEs, we differentiate Equation (4) with respect to $\beta_j$ for $j = 0, \ldots, K$, and find the point $\hat{\beta}$ at which the derivative is equal to zero using a Newton Raphson algorithm. The variance-covariance matrix associated with the estimator $\hat{\beta}$ of $\beta$ is simply the negated inverse of the information matrix. Finding the MLE $\hat{\beta}$ of $\beta$, as well as an estimate of its asymptotic covariance matrix is easily accomplished using a standard statistical software package such as R.[17]

## 3. JOINT MODEL FOR EXCESS AND DEFICIENCY

A JMED can be created using a logistic regression model of the type discussed above. We define the response variable in such a way that it captures whether or not the $i^{\text{th}}$ observation exhibits an adverse health outcome, which can occur as a result

ED_002191_00008780-00003

of an excess or deficient condition. Specifically, we define:

$$Y_i = \begin{cases} 1, \text{ for an adverse response} \\ 0, \text{ for a homeostatic response.} \end{cases}$$

We shall use an indicator variable in the JMED to identify whether an adverse outcome was due to an excess or deficient condition within a given study. To illustrate the development of the JMED, we assume for simplicity that the probability of an adverse response depends only on a single covariate reflecting the level of exposure, and its interaction with the indicator variable described above. In this case, the JMED is given by:

$$P(Y_i = 1) = \frac{\exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i})}{1 + \exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i})}, \quad (5)$$

where $x_{1i}$ is the most appropriate measure of exposure concentration (e.g., untransformed, natural logarithm, or logarithm base 10) for the $i^{\text{th}}$ observation and $x_{2i} = 1$ if the $i^{\text{th}}$ observation is due to excess, and 0 otherwise. Given the type of departure from homeostasis under investigation (excess or deficiency), the JMED above describes the probability of an adverse response at a specified exposure level. The inclusion of an interaction in the JMED permits the relationship between the probability of an adverse response and exposure concentration to be different depending upon the type of departure from homeostasis. Specifically, the JMED describes the probability of an adverse response due to excess exposure as:

$$P(Y_{E_i} = 1) = \frac{\exp[(\beta_0 + \beta_2) + (\beta_1 + \beta_3) x_{1i}]}{1 + \exp[(\beta_0 + \beta_2) + (\beta_1 + \beta_3) x_{1i}]}, \quad (6)$$

and

$$P(Y_{D_i} = 1) = \frac{\exp(\beta_0 + \beta_1 x_{1i})}{1 + \exp(\beta_0 + \beta_1 x_{1i})}, \quad (7)$$

for insufficient exposure (deficiency). Assuming $\beta_1 < 0$, $\beta_3 > 0$, and $\beta_1 + \beta_3 > 0$, a display of $P(Y_{E_i} = 1)$ and $P(Y_{D_i} = 1)$ versus $x_{1i}$ would appear as in Fig. 1.

As will be illustrated in Section 3.3, the JMED can easily be extended to include other covariates.

### 3.1. Equiprobable Crossover Point (EPCP)

The point where the excess and deficiency curves cross corresponds to the level of exposure at which the probabilities of adverse effects due to excess or deficiency are equal and will be referred to here as the EPCP. The EPCP has a closed-form solution since the intersection point between the two logistic regression equations for excess and deficiency can be solved algebraically. Setting Equation (6) equal to Equation (7) and solving for $x_{1i}$ leads to:

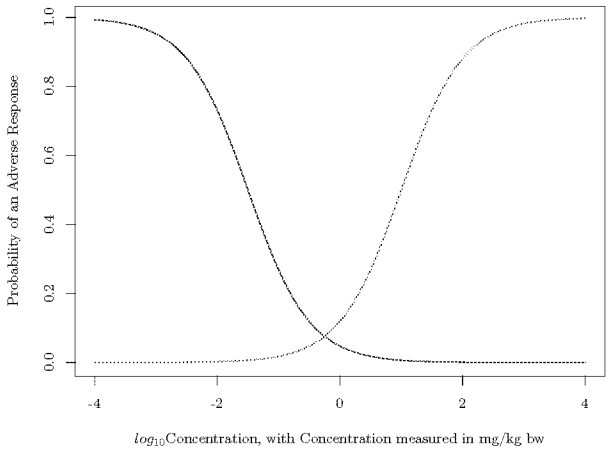


**Fig. 1.** Components of the JMED based on $\log_{10}$ exposure concentration: excess (green) and deficiency (blue) logistic regression curves (colors visible in on-line version).

$$\text{EPCP} = -\frac{\beta_2}{\beta_3}, \quad (8)$$

which is estimated by:

$$\widehat{\text{EPCP}} = -\frac{\hat{\beta}_2}{\hat{\beta}_3}. \quad (9)$$

#### 3.1.1. *Properties of the EPCP*

First- and second-order Taylor series expansions of $-\hat{\beta}_2/\hat{\beta}_3$ about $\hat{\beta}_2 = \beta_2$ and $\hat{\beta}_3 = \beta_3$ can be used to derive approximate expressions for the bias and variance of the estimator, $-\hat{\beta}_2/\hat{\beta}_3$. Considering the general case, $-\hat{\beta}_i/\hat{\beta}_j$, a first-order Taylor series expansion can be employed to obtain an approximate expression for $\text{Var}[-\hat{\beta}_i/\hat{\beta}_j]$, which can by estimated by:

$$\hat{\text{Var}}\left[-\frac{\hat{\beta}_i}{\hat{\beta}_j}\right] \approx \frac{1}{\hat{\beta}_j^2}\hat{\text{Var}}(\hat{\beta}_i) + \frac{\hat{\beta}_i^2}{\hat{\beta}_j^4}\hat{\text{Var}}(\hat{\beta}_j) - \frac{2\hat{\beta}_i}{\hat{\beta}_j^3}\hat{\text{cov}}(\hat{\beta}_i, \hat{\beta}_j) = \hat{C}^T \hat{\Sigma}_{\beta_{(i,j)}} \hat{C}, \quad (10)$$

where $\hat{\Sigma}_{\beta_{(i,j)}}$ is a reduced estimated asymptotic covariance matrix composed of the rows and

ED_002191_00008780-00004

columns of $\hat{\Sigma}_\beta$ associated with $\hat{\beta}_i$ and $\hat{\beta}_j$, and $\hat{C}$ is a $p$-dimensional vector containing the coefficients $-1/\hat{\beta}_j$ and $\hat{\beta}_i/\hat{\beta}_j^{\,2}$ in the first-order Taylor series expansion evaluated at $\beta = \hat{\beta}$, and with the remaining components all zero. The nonzero coefficients are located in $\hat{C}$ based on the relative positions of $\beta_i$ and $\beta_j$ in the logistic regression model. The second-order Taylor series expansion can be employed to propose an approximate expression for Bias$[-\hat{\beta}_i/\hat{\beta}_j]$, which can be estimated by:

$$\widehat{\text{Bias}}\left[-\frac{\hat{\beta}_i}{\hat{\beta}_j}\right] \approx -\frac{\hat{\beta}_i}{\hat{\beta}_j^3}\,\hat{\text{Var}}(\hat{\beta}_j) + \frac{1}{\hat{\beta}_j^2}\,\hat{\text{cov}}(\hat{\beta}_i, \hat{\beta}_j). \quad (11)$$

Assuming for the time being that the bias in the estimator of the EPCP is negligible, Equation (10) may be used to propose a $100(1-\alpha)\%$ large sample confidence interval[15] for $-\beta_i/\beta_j$, namely,

$$-\frac{\hat{\beta}_i}{\hat{\beta}_j} \pm z_{1-\frac{\alpha}{2}}\hat{\text{SE}}\left[-\frac{\hat{\beta}_i}{\hat{\beta}_j}\right], \quad (12)$$

where $\hat{\text{SE}}[-\hat{\beta}_i/\hat{\beta}_j] = \sqrt{\hat{\text{Var}}[-\hat{\beta}_i/\hat{\beta}_j]}$.

### *3.1.2. Odds of an Adverse Response at the EPCP*

The logit function evaluated at the EPCP

$$\text{logit}\,[\pi\,(\text{EPCP})] = \log\left[\frac{\pi\,(\text{EPCP})}{1-\pi\,(\text{EPCP})}\right] \quad (13)$$

represents the log odds of an adverse response at the EPCP. Note that the expression for the log odds evaluated at the EPCP will be identical for the excess and deficiency cases, since this is the point where the two curves intersect. For simplicity, consider the case of a deficiency study when $x_{2i} = 0$. The log odds at the EPCP is:

$$\log\left[\frac{\pi\,(\text{EPCP})}{1-\pi\,(\text{EPCP})}\right] = \beta_0 + \beta_1\left(-\frac{\beta_2}{\beta_3}\right) = \beta_0 - \frac{\beta_1\beta_2}{\beta_3}. \quad (14)$$

Note that the expression for the log odds at the EPCP for an excess study is the same. Using the same approach employed to develop a confidence interval for the EPCP, namely, Taylor series expansions, it is possible to establish approximate expressions for the bias and variance of the estimator of the expression for the log odds in Equation (14).

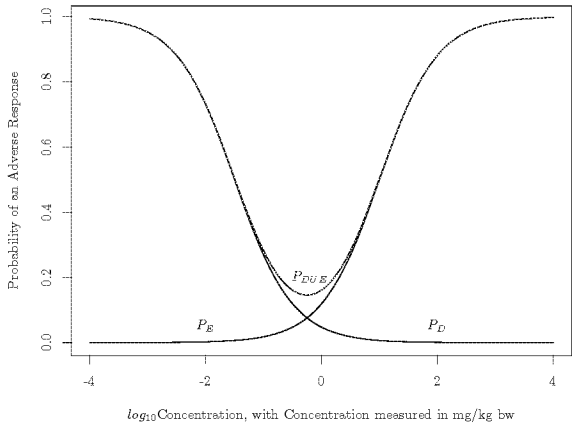


**Fig. 2.** Example of a U-shaped exposure-response curve calculated using the independence model based on $\log_{10}$ exposure concentration.

## 3.2. The Independence Model

The JMED provides a means for estimating the probabilities of the occurrence of excess and deficient conditions, $P(Y_{E_i}=1)$ and $P(Y_{D_i}=1)$ at concentration level $x_{1i}$ using Equations (6) and (7), respectively. The independence model (IM) represents an extension of the JMED, in that it allows for the construction of a single U-shaped curve to describe the excess and deficiency data simultaneously, under the assumption of independence between these two components of the data. If we assume that for a given subject with concentration $x_{1i}$, the presence or absence of an excess condition is independent of a deficient one (and vice versa), we can write the probability that this subject experiences an adverse outcome due to either excess or deficiency, or both, as follows:

$$P_{D\cup E} = P_D + P_E - P_D P_E, \quad (15)$$

where $P_D = P(Y_{D_i} = 1)$ and $P_E = P(Y_{E_i} = 1)$. We refer to Equation (15) as the IM. The IM uses the excess and deficiency probability equations from the JMED, and provides a means of modeling excess and deficiency simultaneously under the assumption of independence of these two outcomes.

### *3.2.1. Investigating $x_{MIN\ D\cup E}$*

Assuming $\beta_1 < 0$ and $\beta_3 > 0$, the graphical representation of $P_{D\cup E}$ is a U-shaped curve, as shown in Fig. 2. Interest lies in finding the exposure level that occurs at the trough of this curve, as this corresponds to the exposure level that minimizes the overall risk due to excess or deficiency. This point will be notated

ED_002191_00008780-00005

as $x_{MIN\ DUE}$. To calculate $x_{MIN\ DUE}$, it is necessary to find the value of $x^*_{1i}$ that minimizes $P_{DUE}$. We set:

$$\frac{\partial P_{DUE}}{\partial x^*_{1i}} = 0,$$

where

$$\frac{\partial P_{DUE}}{\partial x^*_{1i}} = \frac{\partial}{\partial x^*_{1i}}[P_D + P_E - P_D P_E].$$

As there is no closed-form expression for $x^*_{1i}$, this value is obtained numerically using the Newton Raphson algorithm. Since it is not possible to derive a closed-form expression for $x_{MIN\ DUE}$, a bootstrap approach to confidence interval estimation for $x_{MIN\ DUE}$ will be employed. With this approach, B bootstrap samples are generated from the original data set, and $\hat{x}^{(b)}_{MIN\ DUE}$, $b = 1, \ldots B$, is calculated for each. Using these estimates, it is possible to obtain a 95% bootstrap confidence interval for $x_{MIN\ DUE}$, given by:

$$\left(\hat{x}^{(b)}_{MIN\ DUE[0.025]}, \hat{x}^{(b)}_{MIN\ DUE[0.975]}\right),$$

where $\hat{x}^{(b)}_{MIN\ DUE[0.025]}$ and $\hat{x}^{(b)}_{MIN\ DUE[0.975]}$ represent the 2.5th and 97.5th percentiles of the empirical distribution of the B values for $\hat{x}^{(b)}_{MIN\ DUE}$. A similar procedure may also be used to obtain a bootstrap confidence interval for the EPCP, as an alternative to the asymptotic standard normal confidence interval discussed previously.

#### *3.2.2. Odds of an Adverse Response at $x_{MIN\ DUE}$*

The odds of an adverse response occurring at $x_{MIN\ DUE}$ may provide a useful measure of risk in risk assessment applications. Consider the logit function evaluated at $x_{MIN\ DUE}$, which represents an expression for the log odds of an adverse health effect at $x_{MIN\ DUE}$:

$$\text{logit}\,[\pi\,(x_{MIN\ DUE})] = \log\left[\frac{\pi\,(x_{MIN\ DUE})}{1 - \pi\,(x_{MIN\ DUE})}\right].$$

For a deficiency condition, the log odds are:

$$\log\left[\frac{\pi\,(x_{MIN\ DUE})}{1 - \pi\,(x_{MIN\ DUE})}\right] = \beta_0 + \beta_1 x_{MIN\ DUE}, \quad (16)$$

and the corresponding odds are $\exp(\beta_0 + \beta_1 x_{MIN\ DUE})$. Similarly, for an excess condition, the log odds and odds are:

$$\log\left[\frac{\pi\,(x_{MIN\ DUE})}{1 - \pi\,(x_{MIN\ DUE})}\right] = \beta_0 + \beta_2 + (\beta_1 + \beta_3)x_{MIN\ DUE}, \quad (17)$$

and $\exp(\beta_0 + \beta_2 + (\beta_1 + \beta_3)x_{MIN\ DUE})$, respectively. Note that it is possible to derive standard normal confidence intervals for the above log odds quantities using the same asymptotic approach as was used for the EPCP.

### 3.3. Stratification by Species

A natural extension of the JMED is the inclusion of additional variables to account for interspecies differences, thereby creating opportunities for species-specific analyses. In what follows, methods are developed for comparing different species; motivation for this lies in the example presented in Section 5 where data collected on mice, rats, and humans are analyzed. This extension requires the addition of indicator variables to the JMED considered in Equation (5). The characterization below allows rich data from one species to fill in sparse data gaps in another species. For the purpose of the application presented in Section 5, we define:

$$x_{3i} = \begin{cases} 1, \text{ if the } i^{th} \text{ observation is based on a mouse study} \\ 0, \text{ otherwise,} \end{cases}$$

and

$$x_{4i} = \begin{cases} 1, \text{ if the } i^{th} \text{ observation is based on a rat study} \\ 0, \text{ otherwise.} \end{cases}$$

Note that humans are accounted for when $x_{3i} = x_{4i} = 0$. All possible two-way interaction terms will also be included in the JMED, providing species-stratified intercept and slope coefficients for the excess and deficiency curves. This representation facilitates unique excess and deficiency models for humans, rats, and mice. Recall $x_{1i}$ is exposure concentration for the $i^{th}$ observation and $x_{2i} = 1$ if the $i^{th}$ observation is due to excess, and 0 otherwise. The probability of an adverse outcome may be expressed as:

$$P(Y_i = 1) = \frac{\exp\left(\sum_{k=0}^{9} \beta_k x_{ki}\right)}{1 + \exp\left(\sum_{k=0}^{9} \beta_k x_{ki}\right)}, \quad (18)$$

where

$$\sum_{k=0}^{9} x_{ki}\beta_k = \beta_0 x_{0i} + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{4i} + \beta_5 x_{5i} + \beta_6 x_{6i} + \beta_7 x_{7i} + \beta_8 x_{8i} + \beta_9 x_{9i},$$

in which $x_{0i} = 1$, $x_{5i} = x_{1i}x_{2i}$, $x_{6i} = x_{1i}x_{3i}$, $x_{7i} = x_{1i}x_{4i}$, $x_{8i} = x_{2i}x_{3i}$, and $x_{9i} = x_{2i}x_{4i}$. Note that it is not necessary to use the complex model in Equation (18) if

ED_002191_00008780-00006

there is sufficient data available for the species of interest; in this case, the JMED in Equation (5) can be applied separately to data from different species. Inclusion of data from multiple species within the same model may be helpful in the presence of limited data on one or more species, although this will entail assumptions about the relative potency of the agent of interest in different species. Even with adequate data on all species of interest, fitting the JMED to all available data for all species may be of interest in terms of providing an overall parsimonious description of the data for all species.

#### 3.3.1. *Species-Specific EPCPs*

By specifying the species-related indicator variables ($x_{3i}$ and $x_{4i}$) appropriately, we can create exposure-response models for humans, mice, or rats. The exposure-response model for a deficiency study in humans is:

$$P(Y_{D_i} = 1) = \frac{\exp(\beta_0 + \beta_1 x_{1i})}{1 + \exp(\beta_0 + \beta_1 x_{1i})}.$$

For an excess study, the exposure-response model for humans is:

$$P(Y_{E_i} = 1) = \frac{\exp(\beta_0 + \beta_2 + (\beta_1 + \beta_5)x_{1i})}{1 + \exp(\beta_0 + \beta_2 + (\beta_1 + \beta_5)x_{1i})}.$$

Equating $P(Y_{D_i} = 1) = P(Y_{E_i} = 1)$ and solving for $x_{1i}$ provides:

$$EPCP_H = -\frac{\beta_2}{\beta_5}.$$

Similarly, the EPCP for mice is:

$$EPCP_M = -\frac{\beta_2 + \beta_8}{\beta_5},$$

and the EPCP for rats is:

$$EPCP_R = -\frac{\beta_2 + \beta_9}{\beta_5}.$$

It is possible to derive approximate expressions for the bias and variance of the EPCP estimators of these quantities using Taylor series expansions, as demonstrated in Section 3.1.1. Moreover, it is possible to compute the odds of an adverse response and appropriate confidence intervals using the same approach as was employed in Section 3.1.1.

#### 3.3.2. *Species-Specific* $x_{MINDUE}$

The species-stratified JMED gives rise to excess and deficiency exposure-response models for each species. These models can then be used to determine species-specific IMs that permit the identification of $x_{MINDUE}$ for each species. The Newton Raphson algorithm can be used to solve for each $x_{MINDUE}$, while a bootstrap approach may be used to construct a confidence interval for each of the species-specific $x_{MINDUE}$ values. Note that it is also possible to compute the odds of an adverse response at each $x_{MINDUE}$ and a corresponding asymptotic confidence interval. The same approach is employed as in the case of the EPCP; specifically, in developing an expression for the asymptotic variance of the odds at $x_{MINDUE}$, $x_{MINDUE}$ is treated as a constant and entries from the asymptotic covariance matrix are used where required.

## 4. SIMULATION STUDY

To investigate the statistical properties of the methods described in Section 3, a simulation study was conducted to establish their validity. For simplicity, we focus only on the JMED and IM without species stratification.

### 4.1. Simulating the EPCP

Consider the model in Equation (5); namely,

$$\pi_i = P(Y_i = 1) = \frac{\exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i})}{1 + \exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i})},$$

with $x_{1i}$ representing the $\log_{10}$ concentration of the $i^{th}$ observation and $x_{2i}$ an indicator variable for excess or deficiency. Here, $\beta_0 = -2.5$, $\beta_1 = -3.5$, $\beta_2 = 1.5$, and $\beta_3 = 4.5$, yielding EPCP= $-0.33$ logarithm base 10 of the exposure concentration. These values are chosen to be similar to the estimate of the $\beta$ parameter vector obtained when fitting the above model to the data set considered in Section 5. This data set consists of 3,886 observations: 1,943 observations are from deficiency studies and 1,943 are from excess studies. Each observation also had available a log concentration measure and a species identifier.

Initially, we generated a single simulated data set by computing $\pi_i$, given in the model above, for each observation in the actual data set discussed in Section 5 that is available for analysis. For a given

ED_002191_00008780-00007

observation $i$, a simulated response, $Y_i^{(r)}$, is generated from a Bernoulli distribution with parameter $\pi_i$. The generated $Y_i^{(r)}$ along with the values for $x_{1i}$ and $x_{2i}$ in the data set described above were used to fit the model in Equation (5), providing estimates $\hat{\beta}_0^{(r)}, \hat{\beta}_1^{(r)}, \hat{\beta}_2^{(r)}, \hat{\beta}_3^{(r)}$, and an associated estimated asymptotic covariance matrix. These estimates then allowed for the computation of a point estimate of the EPCP, $\widehat{\text{EPCP}}^{(r)}$, along with an approximate 95% large sample confidence interval using the formula in Equation (12). This procedure was repeated 1,000 times, providing 1,000 point estimates of the EPCP and their associated normal confidence intervals. Over the 1,000 replications, the average $\widehat{\text{EPCP}}^{(r)}$ was −0.3269, which compares very favorably to the true EPCP of −0.33. In addition, we determined the coverage rate of the 95% confidence intervals to be 95.4%, which is quite close to the 95% nominal level.

A bootstrap approach could also be used to obtain a confidence interval for EPCP. For example, if we consider the $r^{\text{th}}$ simulated data set described above with associated parameter estimates $\hat{\beta}_0^{(r)}, \hat{\beta}_1^{(r)}, \hat{\beta}_2^{(r)}$, and $\hat{\beta}_3^{(r)}$, we would proceed with computing $\pi_i^{(r)}$ for all $i$ by replacing $\beta_0, \ldots, \beta_3$ in Equation (5) with $\hat{\beta}_0^{(r)}, \hat{\beta}_1^{(r)}, \hat{\beta}_2^{(r)}$, and $\hat{\beta}_3^{(r)}$. For each given observation $i$, a bootstrap response, $Y_i^{(r)(b)}$, is generated from a Bernoulli distribution with parameter $\pi_i^{(r)}$. The generated $Y_i^{(r)(b)}$ along with the values for $x_{1i}$ and $x_{2i}$ in the data set described above are then used to fit the model in Equation (5), providing $\hat{\beta}_0^{(r)(b)}, \hat{\beta}_1^{(r)(b)}, \hat{\beta}_2^{(r)(b)}$, and $\hat{\beta}_3^{(r)(b)}$. These estimates are employed to compute the estimate $\widehat{\text{EPCP}}^{(r)(b)}$. For the $r^{\text{th}}$ simulated data set, if we repeat this procedure for $b = 1, \ldots, B$ bootstrap samples, we can determine $B$ estimates $\widehat{\text{EPCP}}^{(r)(b)}$, which can be used to determine a 95% bootstrap confidence interval for EPCP given by:

$$\left(\widehat{\text{EPCP}}_{[0.025]}^{(r)(b)}, \widehat{\text{EPCP}}_{[0.975]}^{(r)(b)}\right),$$

where $\widehat{\text{EPCP}}_{[0.025]}^{(r)(b)}$ and $\widehat{\text{EPCP}}_{[0.975]}^{(r)(b)}$ represent the $2.5^{\text{th}}$ and $97.5^{\text{th}}$ percentiles of the empirical distribution of the $B$ values for $\widehat{\text{EPCP}}^{(r)(b)}$. For each of the $r = 1, \ldots, 1{,}000$ data sets that we simulated, we generated $b = 1, \ldots, 1,000$ bootstrap samples, using the latter to compute a 95% bootstrap confidence interval for EPCP for each simulated data set. We determined the coverage rate of the bootstrap confidence intervals that covered the true EPCP to be 94.5%, which is very close to the 95% nominal level, and similar to the coverage of the standard normal confidence intervals (95.4%) noted above.

**Table I.** Percent Relative Bias (RB), Relative Standard Deviation (RSD), and Relative Mean Square Error (RMSE) of $\widehat{\text{EPCP}}$ Along with Percent Coverage Rates (CVG) for 95% Standard Normal (SN) and Bootstrap (B) Confidence Intervals for Selected Values of $\beta_3$

| | | | | | CVG (%) | |
|---|---|---|---|---|---|---|
| $\beta_3$ | EPCP | RB (%) | RSD (%) | RMSE (%) | SN | B |
| 0.6 | −1.66 | 2.0 | 8.8 | 8.7 | 95.8 | 95.2 |
| 1 | −1 | 0.2 | 10.3 | 10.3 | 96.1 | 96.1 |
| 1.5 | −0.66 | 0.8 | 11.4 | 11.4 | 95.5 | 94.9 |
| 2 | −0.5 | −0.5 | 13.1 | 13.0 | 94.5 | 94.5 |
| 2.5 | −0.4 | −1.0 | 14.2 | 14.2 | 94.7 | 94.5 |
| 3 | −0.333 | 1.3 | 15.1 | 15.0 | 95.1 | 94.4 |

The approximate variance and bias formulae given in Equations (10) and (11) for the $\widehat{\text{EPCP}}$ are sensitive to the values of $\beta_3$ and Var $[\beta_3]$, respectively. We therefore conducted a simulation study to investigate the impact of the size of $\beta_3$ relative to the other $\beta$ parameters in the model on the bias of $\widehat{\text{EPCP}}$. Specifically, we repeated the simulation described in Section 4.1 where the parameters $\beta_0, \beta_1, \beta_2$ were set to −2, −0.5, and 1, respectively, with different values of $\beta_3$ ranging from 0.6 to 3. These values were selected so that $\beta_1 + \beta_3 > 0$. As indicated in Table I, the relative bias is essentially negligible (less than 2% in all cases considered), with the relative mean squared error being at most 15%.

In calculating the Monte Carlo simulation error, we assume that the coverage rate of the confidence intervals lies within three standard deviations of the nominal level, 95%. Since 1,000 replications were run for each chosen value of $\beta_3$, the Monte Carlo simulation error on the coverage probabilities can be approximated as $3\sqrt{\frac{0.95(0.05)}{1{,}000}} = 0.02$, which indicates that the proportion of intervals that contain the true EPCP should lie between (0.93, 0.97). No evidence of inaccurate coverage is seen for any of the values of $\beta_3$ considered in Table I.

### 4.2. Simulating the $x_{\text{MIN DUE}}$

In order to simulate $x_{\text{MIN DUE}}$ presented in Section 3, we consider the IM given in Equation (15),

ED_002191_00008780-00008

where:

$$P(Y_{E_i} = 1) = \frac{\exp(-1 + x_{1i})}{1 + \exp(-1 + x_{1i})},$$

$$P(Y_{D_i} = 1) = \frac{\exp(-2.5 - 3.5x_{1i})}{1 + \exp(-2.5 - 3.5x_{1i})}.$$

The values of $\beta_0, \beta_1, \beta_2$, and $\beta_3$ are used in the validation procedure for EPCP. The same sample size (1,943 observations on excess, 1,943 observations on deficiency) was also used. The Newton Raphson algorithm was initialized with $x_{1i} = 0.1$ with a convergence criteria of $\epsilon = 10^{-8}$. The algorithm converged at −0.00971661 of the logarithm base 10 concentration, which is the true value of $x_{MIN\ DUE}$ with these parameters.

In order to evaluate the procedures proposed in Section 3 for estimating $x_{MIN\ DUE}$, as was done for EPCP, we generated a single simulated data set using Equation (5) by computing $\pi_i$ for each observation available for analysis in the data set in Section 5. For a given observation, $i$, a simulated response, $Y_i^{(r)}$, is generated from a Bernoulli distribution with parameter $\pi_i$. The generated $Y_i^{(r)}$ along with the values for $x_{1i}$ and $x_{2i}$ were fit to the model in Equation (5), providing $\hat{\beta}_0^{(r)}, \hat{\beta}_1^{(r)}, \hat{\beta}_2^{(r)}$, and $\hat{\beta}_3^{(r)}$. This procedure was repeated 1,000 times, to generate 1,000 simulated $\beta$ parameters. For each of these $r = 1, \ldots 1{,}000$ simulated data sets, we obtained an estimate of $\hat{x}_{MIN\ DUE}^{(r)}$. The average of these 1,000 estimates for $\hat{x}_{MIN\ DUE}^{(r)}$ is −0.0103, which is close to the true value of $x_{MIN\ DUE}$.

Unlike EPCP, it is not possible to obtain a closed-form expression for $x_{MIN\ DUE}$. Thus, in order to determine a confidence interval for the latter quantity, a bootstrap technique analogous to the one used for the EPCP was employed. Suppose we consider one of the 1,000 simulated data sets described above with associated parameter estimates $\hat{\beta}_0^{(r)}, \hat{\beta}_1^{(r)}, \hat{\beta}_2^{(r)}$, and $\hat{\beta}_3^{(r)}$. We may proceed by computing $\pi_i^{(r)}$. For each observation, $i$, a bootstrap response, $Y_i^{(r)(b)}$, is generated from a Bernoulli distribution with parameter $\pi_i^{(r)}$. The model in Equation (5) was fit to the generated $Y_i^{(r)(b)}$, along with the values for $x_{1i}$ and $x_{2i}$, providing $\hat{\beta}_0^{(r)(b)}, \hat{\beta}_1^{(r)(b)}, \hat{\beta}_2^{(r)(b)}$, and $\hat{\beta}_3^{(r)(b)}$. To obtain one bootstrap estimate of $x_{MIN\ DUE}$, denoted as $\hat{x}_{MIN\ DUE}^{(r)(b)}$, we applied the Newton Raphson algorithm to minimize $P_{DUE}$ evaluated at $\hat{\beta}_0^{(r)(b)}, \hat{\beta}_1^{(r)(b)}, \hat{\beta}_2^{(r)(b)}$, and $\hat{\beta}_3^{(r)(b)}$. Within one simulated data set, we generated 1,000 bootstrap samples, and determined $\hat{x}_{MIN\ DUE}^{(r)(b)}$, $b = 1, \ldots 1{,}000$ for each bootstrap sample. Using these estimates, we can obtain a 95% bootstrap confidence interval for $x_{MIN\ DUE}$, given by:

$$\left(\hat{x}_{MIN\ DUE[0.025]}^{(r)(b)}, \hat{x}_{MIN\ DUE[0.975]}^{(r)(b)}\right),$$

where $\hat{x}_{MIN\ DUE[0.025]}^{(r)(b)}$ and $\hat{x}_{MIN\ DUE[0.975]}^{(r)(b)}$ represent the 2.5th and 97.5th percentiles of the empirical distribution of the 1,000 values for $\hat{x}_{MIN\ DUE}^{(r)(b)}$. For each of the $r = 1, \ldots,$ 1,000 data sets that we simulated, we generated $b = 1, \ldots,$ 1,000 bootstrap samples, and computed a 95% bootstrap confidence interval for $x_{MIN\ DUE}$ for each simulated data set. We determined the coverage rate of these 95% bootstrap confidence intervals that cover $x_{MIN\ DUE}$ to be 94.2%, which is very close to the 95% nominal value.

**Table II.** Percent Relative Bias (RB), Standard Deviation (RSD), and Mean Square Error (RMSE) of $\hat{x}_{MIN\ DUE}$ Along with Percent Coverage Rates (CVG) for 95 % Bootstrap Confidence Intervals for Selected Values of $\beta_3$

| $\beta_3$ | $x_{MIN_{DUE}}$ | RB (%) | RSD (%) | RMSE (%) | CVG (%) |
|---|---|---|---|---|---|
| 0.6 | −0.12 | 15.5 | 140.8 | 141.6 | 96.7 |
| 1 | −1.00 | 3.5 | 25.5 | 25.7 | 96.2 |
| 1.5 | −1.06 | 1.5 | 11.5 | 11.6 | 97.0 |
| 2 | −0.90 | −0.02 | 8.4 | 8.4 | 93.0 |
| 2.5 | −0.75 | 0.3 | 8.1 | 8.1 | 92.0 |
| 3 | −0.64 | −0.2 | 8.5 | 8.5 | 94.0 |

The results as summarized in Table II indicate no significant concerns with respect to bias or coverage probability.

## 5. DATA EXAMPLE

### 5.1. Application to a Copper Toxicity Database

After completing a detailed literature review and applying appropriate inclusion/exclusion criteria, Krewski *et al.*[10] developed a comprehensive Cu toxicity database designed for the application of categorical regression. Relevant information, including species, age, sex, route of exposure, concentration, and duration of exposure, was abstracted from each study and stored in a computerized database. Adverse health outcomes reported in each study were noted and severity scores were assigned to each. The elaboration of the severity scoring system for copper excess and deficiency summarized in Table III was

ED_002191_00008780-00009

**Table III.** Thirteen-Level Severity Scoring System for Copper Excess and Deficiency

| Outcome | Severity Score ($S$) | Physiological Response |
|---|---|---|
| | −6 | Death |
| | −5 | Serious irreversible gross deficiency |
| Deficiency | −4 | Reversible gross deficiency |
| | −3 | Metabolic perturbation |
| | −2 | Early biological indicators of deficient Cu levels |
| | −1 | Homeostatic adaptation to low intakes |
| Homeostasis | 0 | No effect |
| | 1 | Homeostatic adaptation to high intakes |
| | 2 | Early biological indicators of accumulated Cu |
| | 3 | Metabolic perturbation |
| Excess | 4 | Reversible gross excess |
| | 5 | Serious irreversible gross excess |
| | 6 | Death |

guided by a detailed review of indicators of toxicity from excess and deficiency.[12]

Chambers *et al.*[8] made modifications to the Cu toxicity database in preparation for their categorical regression analysis. These modifications include a redefined dose metric to allow for interspecies differences related to body weight, as well as conservatively using the most severe health effect to represent an entire exposure group. To facilitate the application of binary logistic regression for our analysis, two further modifications were made. The first modification involved converting data from group level to individual level. Chambers *et al.*[8] performed categorical regression using group-level data; each entry in their copper database corresponded to one experimental group. In our analysis, group-level data were converted to individual-level data by replicating each group-level observation based on the size of each experimental group. Each subject in the experimental group was assigned the same severity score. The second modification involved dichotomizing the ordinal response variable $S$, which represents the severity of the outcome for either excess or deficiency. This dichotomous parameterization is given by:

$$Y_i = \begin{cases} 1, & \text{for } |S| \geq 2 \\ 0, & \text{for } |S| \leq 1. \end{cases}$$

A 13-point severity matrix was developed and applied to the copper database. We investigated the effect of the choice of the cut-off severity score in the parameterization of the response variable for the JMED and found the JMED to be robust against the choice of the cut-off score. For example, if $|S| \leq 2$ had been selected as the cut-off point, the estimates for EPCP and $x_{\text{MIN DUE}}$ (0.46 and 0.98 mg/kg bw/day) are nearly identical to those obtained when $|S| \leq 1$ (0.47 and 0.98 mg/kg bw/day). However, notably different results were obtained for $|S| \leq 3$ (0.14 and 0.1 mg/kg bw/day for the EPCP and $x_{\text{MIN DUE}}$, respectively). The Cu toxicity database contains a large collection of independent variables available for analysis, including categorical (species, age, and sex) and continuous (concentration and duration) covariates. Although different transformations of concentration could be considered and explored, preference was given to the logarithm base 10 transformation since Chambers *et al.*[8] and others[6,7] have chosen this exposure metric in similar work. The effect of duration was not significant, and species was the only significant categorical variable. Although an extensive series of sensitivity analyses would be of value in exploring the effects of different analytic decisions, we will confine our attention here to a more limited, illustrative application of the new methods of exposure-response analysis for a U-shaped exposure-response relationships based on the modeling techniques introduced in this article.

To illustrate the application of the methodology developed in Section 3, we consider a subset of the data composed of 1,943 deficiency observations and 1,943 excess observations. All available deficiency observations were used in the analysis. To provide equally sized excess and deficiency data sets, 1,943 excess observations were randomly selected from all 3,300 available excess observations. We chose this subset so that the excess and deficiency data carried equal weight in the analysis. Within the deficiency data set, there are 131, 368, and 1,444 human, mouse, and rat observations, respectively. Within the excess data set, there are 769, 322, and 852 human, mouse, and rat observations, respectively.

### 5.2. Representations of the JMED

Fitting the JMED in Equation (5) to the data set of 3,886 observations provides estimates of $\beta_0$, $\beta_1$, $\beta_2$, and $\beta_3$, equal to −2.5, −3.5, 1.5, and 4.5, respectively, along with an associated estimated covariance matrix. The JMED provided a significant

ED_002191_00008780-00010

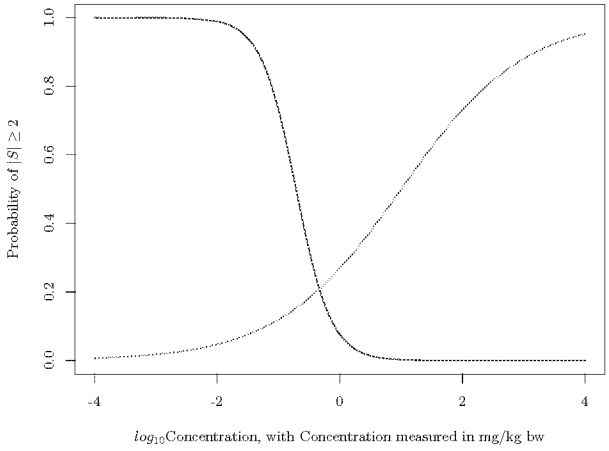


**Fig. 3.** Components of the JMED for all species in the Cu toxicity database: excess (green) and deficiency (blue) logistic regression curves.

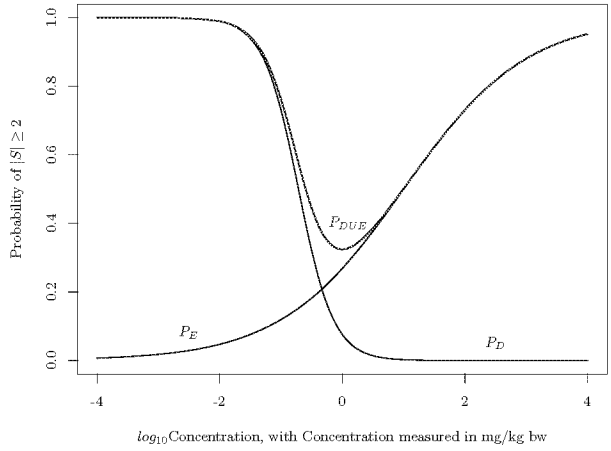


**Fig. 4.** The U-shaped curve generated by modeling $P_{DUE}$ under the IM for all species in the Cu toxicity database.

improvement in fit compared to the null model ($p < 0.001$ based on the likelihood ratio test); all regression coefficients were significantly different from zero ($p < 0.001$ based on the Wald statistic). A plot of the resulting excess and deficiency curves resulting from this fit is given in Fig. 3.

### 5.3. Estimating the EPCP

The EPCP is estimated as −0.33 of the logarithm base 10 transformed concentration, with concentration being measured in mg/kg bw. Thus, at an exposure level of 0.47 mg/kg bw, adverse health effects of specified severity attributed to excess or deficient amounts of Cu are equally likely. Equation (12) facilitates the construction of a 95% confidence interval for the EPCP, which suggests that the concentration at which the probabilities of an adverse effect due to excess and deficiency are equal lies between 0.42 and 0.53 mg/kg bw. However, species differences complicate the utility of this estimate in making inferences about human risk.

#### *5.3.1. Odds of an Adverse Response at the EPCP*

Using Equation (14), the log odds of an adverse response at the EPCP are estimated as −1.4, with the corresponding odds being 0.25. Thus, at the EPCP, a severity score $|S| \geq 2$ will occur 33% of the time, while the complementary response $|S| < 2$ will occur 67% of the time. The occurrence of a homeostatic response is clearly favored at the EPCP.

### 5.4. Results from the Independence Model: $x_{MIN\ DUE}$

Using the JMED fit in Equation (5) along with the assumption of independence between toxicity due to excess and deficiency allows for the estimation of $P_{DUE}$ over the range of exposure levels of interest, represented by logarithm base 10 concentration (Figs. 3 and 4). Using the Newton Raphson method, $x_{MIN\ DUE}$ is estimated as −0.01 of the logarithm base 10 transformed concentration, with concentration being measured in mg/kg bw. For all species, a dose of 0.98 mg/kg bw will minimize the likelihood of an adverse response, or maximize the likelihood of a homeostatic response, emerging from an excess or deficiency condition, or both. Using Equation (15), it is possible to construct a 95% bootstrap confidence interval for $x_{MIN\ DUE}$. Transforming the upper and lower limits of this interval allows us to conclude with 95% confidence that $x_{MIN\ DUE}$ lies between 0.82 and 1.04 mg/kg bw.

#### *5.4.1. Odds of an Adverse Response at $x_{MIN\ DUE}$*

The odds of a severity score $|S| \geq 2$ attributed to excess at $x_{MIN\ DUE}$ are estimated at 0.35; for deficiency, the odds are 0.084. Thus, for a concentration level of 0.98 mg/kg bw, the chances of realizing such a severity score for excess and deficiency are 55% and 11%, respectively.

ED_002191_00008780-00011

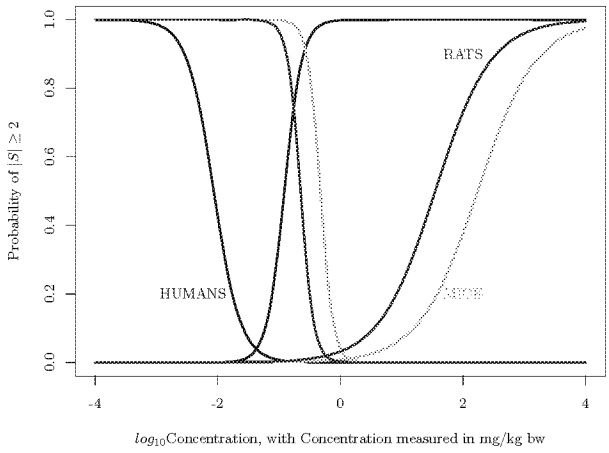


**Fig. 5.** Components of the species-stratified JMED for the Cu toxicity database: excess and deficiency logistic regression curves for humans, mice, and rats.

### 5.5. Stratification by Species

After the Cu toxicity database was expanded to convert group-level to individual-level data, studies conducted on mice and rats comprised an overwhelming proportion of the database. To perform a regression analysis with stratification by species, it was necessary to remove replicates created when converting group-level to individual-level data, so as to ensure the independence of observations in the data set to be analyzed. This provided a data set with 341 observations. As an initial attempt to fit the JMED to the somewhat limited human data alone did not converge, the mice and rat data were modeled together with the human data to accommodate the gaps in the human data and facilitate convergence. Fitting the JMED in Equation (18) to the individual human, rat, and mice data described above yields the regression coefficients $\beta_0, \beta_1, \beta_2, \beta_3, \ldots, \beta_9$ estimated by −9.5, −4.6, 15.9, 6.4, 3.4, 11.6, −4.9, −4.8, −17.5, and −13.2, respectively, along with an associated estimated covariance matrix.

A plot of the excess and deficiency curves for each species is provided in Fig. 5. The human excess and deficiency curves are leftmost in this figure, indicating that humans are most sensitive of the three species considered, followed by rats, then mice.

#### 5.5.1. *Species-Specific EPCPs*

Point estimates and 95% standard normal confidence intervals for species-specific EPCPs are summarized in Table IV. The EPCP for humans is estimated as −1.37 of the logarithm base 10 transformed concentration, with concentration being measured in mg/kg bw; similarly, the EPCPs for mice and rats are estimated to be 0.13 and −0.24, respectively. At exposure levels of 0.042, 1.4, and 0.57 mg/kg bw, the probability of experiencing an adverse response due to excess Cu exposure is equivalent to the probability of experiencing an adverse response due to Cu deficiency for humans, mice, and rats, respectively. With 95% confidence, the EPCP lies between 0.001 and 0.21 mg/kg bw for humans, 0.13 and 14.1 mg/kg bw for mice, and 0.04 and 7.7 mg/kg bw for rats. Assuming mice weigh 20 g and rats weigh 400 g, the estimates for EPCP are 0.016 and 0.39 mg/day, respectively, with confidence limits 0.003 to 0.28 mg/day and 0.016 to 3.08 mg/day.

**Table IV.** Point Estimates and 95% Confidence Intervals for the Species-Specific EPCP's

| Species | $\widehat{\text{EPCP}}$ of $\log_{10}$ Concentration | $\widehat{\text{EPCP}}$ (mg/kg bw) | 95% Std Normal CI |
|---|---|---|---|
| Humans | −1.37 | 0.042 | (0.001, 0.2) |
| Mice | 0.13 | 1.4 | (0.13, 14.1) |
| Rats | −0.24 | 0.57 | (0.04, 7.7) |

Our primary interest is in the results for humans. The point estimate for EPCP, 0.042 mg/kg bw, may be understood as the exposure level that provides an equal chance for Cu toxicity due to either excess or deficiency. In addition, its confidence interval, 0.001 to 0.21 mg/kg bw, may be interpreted as a range of exposure levels that is 95% likely to provide equiprobability of Cu excess and deficiency. For a human who weighs 70 kg, this point estimate of the EPCP transforms to 2.94 mg/day, with confidence limits ranging from 0.07 to 14.7 mg/day.

#### 5.5.2. *Odds of an Adverse Response at the Human EPCP*

For humans, the odds of $|S| \geq 2$ at an intake level of 2.94 mg/day are estimated to be 0.045, translating to a 4.7% chance of an adverse response and a 95.3% chance of a homeostatic one. While the odds favor a homeostatic response, the probability of an adverse response at this exposure level appears somewhat high, reflecting a nontrivial risk due to either excess or deficiency at the EPCP. For mice and rats, the odds of an adverse response may be computed

ED_002191_00008780-00012

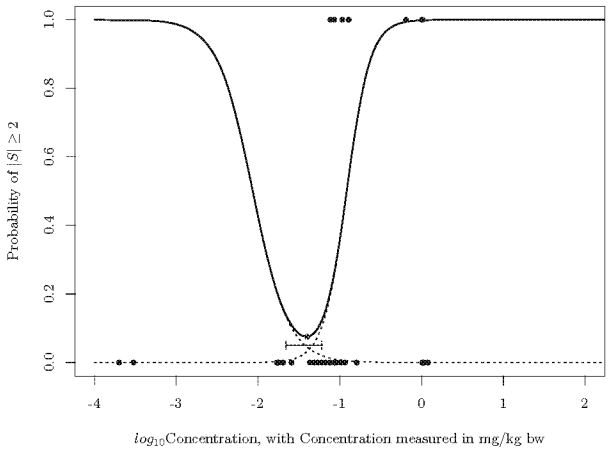


**Fig. 6.** A U-shaped exposure-response curve for humans calculated from the species-stratified JMED and IMs. The estimate for $x_{MIN\ DUE}$ is indicated by a red dot and its 95% bootstrap confidence interval is indicated by the red line segment. The human observations used to fit this model are indicated in blue. Raw data are denoted by blue circles.

using the same approach described above (data not shown).

### 5.5.3. *Species-Specific* $x_{MIN\ DUE}$

The JMED fit in Equation (18) allows for the estimation of $P_{DUE}$ over the range of exposure levels for humans, rats, and mice. A plot of the human U-shaped exposure-response curve superimposed on the excess and deficiency curves is presented in Fig. 6. The bootstrap confidence interval and observations used in the regression analysis are also shown. The estimates of $x_{MIN\ DUE}$ of the logarithm base 10 transformed concentration for humans, mice, and rats, along with their 95% bootstrap confidence intervals, are summarized in Table V. Assuming mice weigh 20 g and rats 400 g, the estimates of $x_{MIN\ DUE}$ are 0.02 and 0.48 mg/day for these two species, respectively, with confidence limits 0.018 to 0.028 mg/day for mice and 0.456 to 0.81 mg/day for rats.

**Table V.** Point Estimates and 95% Confidence Intervals for the Species-Specific $x_{MIN\ DUE}$'s

| Species | $\widehat{x}_{MIN\ DUE}$ of $\log_{10}$ Concentration | $\widehat{x}_{MIN\ DUE}$ (mg/kg bw) | 95% Bootstrap CI |
|---|---|---|---|
| Humans | −1.4 | 0.039 | (0.02, 0.06) |
| Mice | 0.2 | 1.62 | (1.51, 2.07) |
| Rats | −0.16 | 0.69 | (0.57, 0.81) |

Note that bootstrapping was not necessary to calculate 95% standard normal confidence intervals for species EPCPs presented in Section 5.5.1. While computing 95% bootstrap CIs for species $x_{MIN\ DUE}$, we discovered that approximately 25% of the fitted bootstrap models yield a warning message. To move forward with analyses, any fitted bootstrap or replicate model that caused a warning message was excluded from calculations. Although there were no convergence issues, this warning message indicates that there is a concentration threshold that perfectly separates the data. Peduzzi *et al.*[19] employed a similar approach in their work on the number of events per variable in logistic regression analysis, where the authors excluded problematic simulations.

The point estimate for $x_{MIN\ DUE}$, 0.039 mg/kg bw, for humans may be interpreted as the exposure level that minimizes the overall risk of an adverse response due to either excess or deficiency or both. In addition, its bootstrap interval, 0.022 to 0.064 mg/kg bw, may be interpreted as a range of exposure levels that is 95% likely to minimize the overall risk from excess or deficiency. For a human who weighs 70 kg, this point estimate transforms to 2.73 mg/day with a 95% confidence limit of 1.54 to 4.48 mg/day.

Note that a bootstrap approach may also be employed to calculate a 95% confidence interval for the EPCP by filtering all fitted models that induce a warning message. For example, a 95% bootstrap confidence interval for EPCP is 1.02 to 6.68 mg/day.

### 5.5.4. *Odds of an Adverse Response at the Human* $x_{MIN\ DUE}$

The odds of an adverse response at an intake level of 2.73 mg/day attributed to excess and deficiency are estimated by 0.036 and 0.05, respectively, providing 3.7% and 5.5% chances of a health effect of $|S| \geq 2$.

## 6. DISCUSSION

This article focuses on the simultaneous modeling of excess and deficiency to characterize the observed U-shaped exposure-response curve for Cu. The IM achieves this objective, using the JMED as the foundation. In fitting these models to the copper toxicity database studied by Chambers *et al.*,[8] a U-shaped curve was created from the IM when the probability of an adverse health outcome of severity $|S| \geq 2$ is plotted against the logarithm of exposure concentration. The IM is derived from the

ED_002191_00008780-00013

JMED with the additional assumption of statistically independent responses due to excess and deficiency.

Two benchmark exposure levels of interest, specifically, the EPCP and $x_{MIN\ DUE}$, were investigated. The EPCP reflects the exposure concentration at which the risk of an adverse outcome due to excess is equal to that due to deficiency under the JMED; $x_{MIN\ DUE}$ reflects the exposure concentration at which the total risk of an adverse outcome due to either excess or deficiency, or both, is minimized, under the IM. By definition, the JMED and consequentially the EPCP consider excess and deficiency as separate sources of risk. The IM builds on the JMED by accounting for excess and deficiency together; the resulting risk estimate, $x_{MIN\ DUE}$, minimizes risk from both excess and deficiency. As a benchmark for human exposure guidelines, the $x_{MIN\ DUE}$ is therefore preferable to the EPCP. Nonetheless, the EPCP may be a useful indicator of risk in its own right, and provides an alternative benchmark if the independence assumption underlying $x_{MIN\ DUE}$ is questionable.

The inclusion of indicator variables for mice and rats facilitated species-specific analyses of the JMED and IM. By adjusting for differences in species sensitivity, it is possible to conduct combined categorical regression analyses of severity scored data from multiple species, thereby allowing data from other species to inform the development of human exposure guidelines for agents such as copper that demonstrate U-shaped exposure-response relationships. The categorical regression models considered only a limited number of covariates, such as species, gender, and level of exposure. Application of the JMED and IM involving additional covariates such as duration of exposure is straightforward, thereby permitting the fitting of more complex multivariable models when the data permit. In the future, extensions to the categorical regression modeling techniques introduced in this article may be developed, including, for example, the incorporation of random effects to model possibly correlated data.[20]

The Agency for Toxic Substances and Disease Registry (ATSDR) estimates humans receive between 1.0 and 1.3 mg Cu/day from food intake and has identified a minimum risk level (MRL) of 0.7 mg Cu/day.[18] An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects over a specified duration of exposure.[16] The Institute of Medicine (IOM) has established a recommended dietary allowance (RDA) and upper intake level of 0.9 mg Cu/day and 10 mg Cu/day, respectively. The methods presented in this article were developed for agents known to exhibit toxicity for both excess and deficiency; since the copper database was simply an application of the methods presented herein, we had not intended that EPCP and $x_{MIN\ DUE}$ based on the JMED and IM be directly compared to the RDA level established by the IOM. However, the $x_{MIN\ DUE}$ and EPCP risk estimates, along with their confidence intervals, produced by the JMED and IM align well with current human exposure guidelines, demonstrating the promise of this new approach to modeling U-shaped exposure-response relationships. The value of $x_{MIN\ DUE}$ based on the IM could be considered as the optimal intake level, minimizing the joint risks associated with excess and deficiency. For a human who weighs 70 kg, the $x_{MIN\ DUE}$ is 2.7 mg Cu/day. An intake level of 2.7 mg Cu/day minimizes the overall risks attributed to either excess or deficiency (or both). Our results suggest that no level of copper intake is without some risk. This arises because there are offsetting risks associated with either excess or deficient intake. At the optimal estimated intake, 2.7 mg Cu/day, the probability of evidence for biochemical changes being noted in test subjects is 3.7% due to this level being too high for some subjects and 5.6% due to it being too low in other subjects. Although increasing intake would reduce the risk from a low copper intake, this would also lead to an increased risk due to the higher copper intake. The clinical and long-term health implications of these observations require further research. In particular, it would be useful to explore the long-term impact of slightly low or high copper intake in a large sample size, with long-term observations. The analyses could be adjusted to include a weighting for the severity of the outcomes that would lead to an adjusted optimal intake.

Rather than focus entirely on a single optimal intake level, it may be more appropriate to consider an acceptable range of copper intake, allowing for uncertainties in the data and interindividual variability in the response. One possible approach to identifying a reasonable range of exposures would be to base the range on confidence limits around $x_{MIN\ DUE}$. To avoid asymptotic approximation, a bootstrap confidence interval procedure, which is based on the finite sample distribution, is preferred. Although not considered in the present work, another approach to computing confidence intervals could be based on the asymptotic chi-square distribution of the likelihood ratio. Our 95% bootstrap confidence interval on $x_{MIN\ DUE}$ suggests an acceptable range of oral intake between 1.54 and 4.48 mg Cu/day, based on the IM. This is compatible to previous work by

ED_002191_00008780-00014

Chambers *et al.*,[8] using the separate models for excess and deficiency, where the single optimal intake level for humans was estimated to be 2.6 mg Cu/day with uncertainty limits ranging from 1.8 to 3.1 mg Cu/day.

In conclusion, the JMED and IM presented here appear to offer considerable promise in describing U-shaped exposure-response relationships for essential elements such as copper. These models can be used to derive human exposure guidelines, expressed in the form of an optimal intake level, $x_{MIN\ DUE}$, or as a range of allowable oral intakes. The categorical regression techniques used in fitting these models permit a combined analysis of multiple endpoints from multiple studies using a severity scoring system to place diverse endpoints on a common scale. Chemicals with a smaller knowledge base may not have the information to support such a detailed severity scoring system. The construction of severity categories will depend on the toxicological endpoints of interest. If severity categories are not well separated, it may be preferable to use a smaller number of categories.[10] Complex models that incorporate stratification by species, gender, or other factors may be used when the data permit. Data from multiple species can also be used to augment the available human data, with appropriate adjustments for species differences in sensitivity to the agent of interest. Further experience in the application of this technique to agents other than copper—the example chosen for presentation here because of the availability of a comprehensive copper toxicity database—would serve to explore the utility of the modeling methods introduced here across a range of agents that can lead to toxicity in humans due to both excess and deficiency.

## ACKNOWLEDGMENTS

This research was supported in part by Discovery Grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to P. Farrell and D. Krewski. The authors are grateful to two referees for many helpful comments that improved the presentation of the results in this article. D. Krewski is the NSERC Chair in Risk Science at the University of Ottawa.

## REFERENCES

1. Food and Nutrition Board. Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Washington, DC: National Academy Press, 2001.
2. Barnes D, Dourson M. Reference dose (RfD): Description an use in health risk assessments. Regulatory Toxicology and Pharmacology, 1988; 8:471–486.
3. Crump KS. A new method for determining allowable daily intake. Fundamental and Applied Toxicology, 1984; 4:854–471.
4. European Chemicals Agency. Guidance for Human Health Risk Assessment. Volume III, Part B. Version 1.0, December 2013.
5. Sand S, Portier CJ, Krewski D. A signal-to-noise crossover dose as the point of departure for health risk assessment. Environmental Health Perspectives, 2011; 119:1766–1774.
6. Gift JS, McGaughy R, Singh DV, Sonawane B. Health assessment of phosgene: Approaches for derivation of reference concentration. Regulatory Toxicology and Pharmacology, 2008; 51:98–107.
7. Allen B, Zeiger E, Lawrence G, Friedman M, Shipp A. Dose-response modeling of in vivo genotoxicity data for use in risk assessment: Some approaches illustrated by an analysis of acrylamide. Regulatory Toxicology and Pharmacology, 2005; 41(1):6–27.
8. Chambers A, Krewski D, Birkett N, Plunkett L, Hertzberg R, Danzeisen R, Aggett PJ, Starr TB, Baker S, Dourson M, Jones P, Keen CL, Meek B, Schoeny R, Slob W. An exposure-response curve for copper excess and deficiency. Journal of Toxicology and Environmental Health, 2010; 13(7):546–578.
9. CatReg Software User Manual (R-Version). Washington, DC: Office of Research and Development, August 2006. Available at: oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=500572.
10. Krewski D, Chambers A, Stern BR, Aggett PJ, Plunkett L, Rudenko L. Development of a copper database for exposure-response analysis. Journal of Toxicology and Environmental Health, 2010; 73:208–216.
11. Haber L, Strickland JA, Guth DJ. Categorical regression analysis of toxicity data. Comments on Toxicology, 2001; 7(5–6):437–452.
12. Stern BR, Solioz, M, Krewski, D, Aggett, P, Aw, T, Baker, S, Dourson, M, Haber, L, Hertzberg, R, Keen, C, Meek, B, Rudenko, L, Schoeny, R, Slob, W, Starr, T. Copper and human health: Biochemistry, genetics, and strategies for modeling dose-response relationships. A practical approach to microarray data analysis. Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 2007; 10(3):157–222, 437–452.
13. Edler L, Poirier K, Dourson M, Kleiner J, Mileson B, Nordmann H, Renwick A, Slob W, Walton K, Wurtzen G. Mathematical modelling and quantitative methods. Food and Chemical Toxicology, 2002; 40:283–326.
14. Agresti, A. Categorical Data Analysis, 3rd ed. New Jersey: Wiley Series in Probability and Statistics, 2013.
15. Fahrmeir L, Kaufmann H. Consistency and asymptotic normality of the maximum likelihood estimator in genearlized linear models. Annals of Statistics, 1985; 13(1):342–368.
16. Dourson ML, Teuschler LK, Durkin PR, Stiteler WM. Categorical regression of toxicity data: A case study using Aldicarb. Regulatory Toxicology and Pharmacology, 1997; 25:121–129.
17. www.r-project.org/, Accessed January 27, 2016.
18. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cu. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=206tid=37, Accessed June 3, 2015.
19. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. Journal of Clinical Epidemiology, 1996; 49(12):1373–1379.
20. Tooze JA, Midthune D, Dodd KW, Freedman LS, Krebs-Smith SM, Subar AF, Guenther PM, Carroll RJ, Kipnis V. A new method for estimating the usual intake of episodically-consumed foods with application to their distribution. Journal of the American Dietetic Association, 2006; 106(10): 1575–1587.

ED_002191_00008780-00015